

Figure 1

	
	10 20 30 40 50	
Hordeum vulgare	---MASD-HR RFVLSGAVLL SVLAVAAATL E-----	SVKDECQLGV
Oryza sativa	---MASN-KV VFSVLLLAUV SVLAATATMA EYHHQDQVVY	TPGPICQPGM
Hordeum spontaneum	---MAFK--Y QLLLSAAVML AILAATVT--	SFGDMCAPGD
Eleusine coracana	-----	SVGTSCIPGM
Secale cereale	-----	SVGGQCVPGL
Triticum durum	---MACKSSC SLLLLAAVLL SVLAA--A--	SASGSCVPGV
Zea mays	MASSSSSSHR RLILAAVLL SVLAAASA--	SAGTSCVPGW
Triticum aestivum	---MASN-HR RFLLSGAVLL SVLAAVAA-L E-----	SVEDECQPGV
	
	60 70 80 90 100	
Hordeum vulgare	DFPHNPLATC HTYVIKRVCG ---RGPSRPM LV-----	-----KERC
Oryza sativa	GYPMYPLPRC RALVKRQCVG ---RGTAATA EQ-----	-----VRRDC
Hordeum spontaneum	ALPANPLRAC RTYVVSQIC- --HVGPRLLST WD-----	-----MKRRC
Eleusine coracana	AIPHNPLDSC RYVVAKRACG ---VGPRLAT QE-----	-----MKARC
Secale cereale	AMPHNPLGAC RTYVVSQIC- --HVGPRLLFT WD-----	-----MKRRC
Triticum durum	AFRTNLLPHC RDYVLQQTG TFPTGSKLPE WMTSASIYSP	GKPYLAKLYC
Zea mays	AIPHNPLPSC RYVVTSTRTG ---IGPRLPW PE-----	-----LKRRC
Triticum aestivum	AFPHNALATC HTYVIKRVCG ---RGPSRPM LV-----	-----KERC
	
	110 120 130 140 150	
Hordeum vulgare	CRELAAMP-D HCRCEALRIL MDGVRTPE-- ---GRVVEG	RLGDRRDCPR
Oryza sativa	CRQLAAVDDS WCRCEAISHM LGGIYRELG- ---APDVGHP	MSEVFRGCRR
Hordeum spontaneum	CDELSAIP-A YCRCEALRII MDGTVTWQ-- ---GVFG-A	YFKDMPNCPR
Eleusine coracana	CRQLEAIP-A YCRCEAVRIL MDGVVTP--- ---SGQHEGR	LLQDLPGCPR
Secale cereale	CDELLAIP-A YCRCEALRIL MDGVVTQQ-- ---GVFEGG	YLKDMPNCPR
Triticum durum	CQELAEIS-Q QCRCEALRYF IALPVPSQPV DPRSGNVGES	GLIDLPGCPR
Zea mays	CRELADIP-A YCRCTALSIL MDGAIPPGP- ---DAQLEGR	-LEDLPGCPR
Triticum aestivum	CRELAVP-D YCRCEALRVL MDGVRAEE-- ---GHVVEG	RLGDRRDCPR
	
	160 170 180 190	
Hordeum vulgare	EEQRAFAATL VTAAECNLSS VQAPGVRLVL LADG-----	---
Oryza sativa	GDLERAAASL PAFCNVLDIPN GGG-GVCYWL ARSGY-----	---
Hordeum spontaneum	VMQTSYAANL VNPQECNLWT IHGSPSCPEL QPGYEVVL--	---
Eleusine coracana	QVQRAFAPKL VTEVECNLAT IHGGPFCLSL LGAGE-----	---
Secale cereale	VTQRSYAATL VAPQECNLPT IHGSPYCPTL QAGY-----	---
Triticum durum	EMQWDFVRL L VAPGQCNLAT IHNVRYPVAV EQPLWI----	---
Zea mays	EVQRGFAATL VTEAECNLAT ISGVAECPIWI LGGGTMPK--	---
Triticum aestivum	EAQREFAATL VTAAECNLPT VS--GVGSTL GATGRWMTIE	LPK

Figure 2

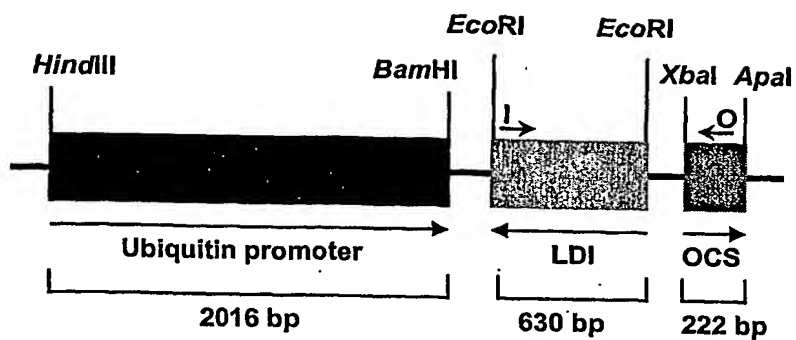


Figure 3

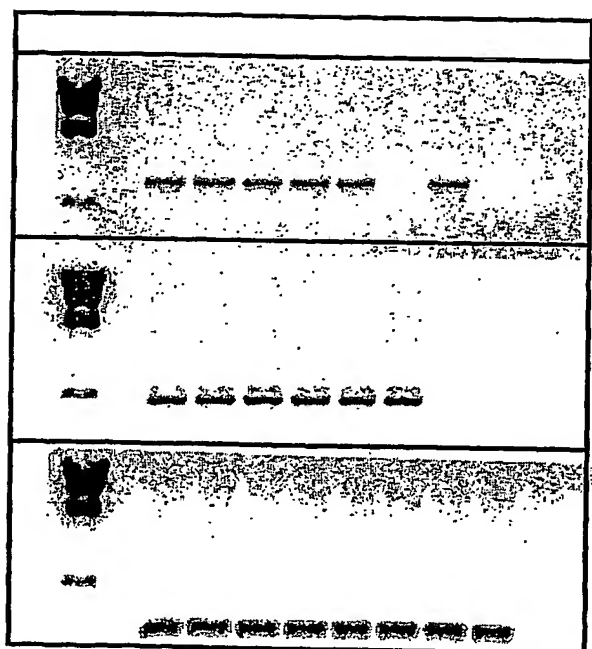
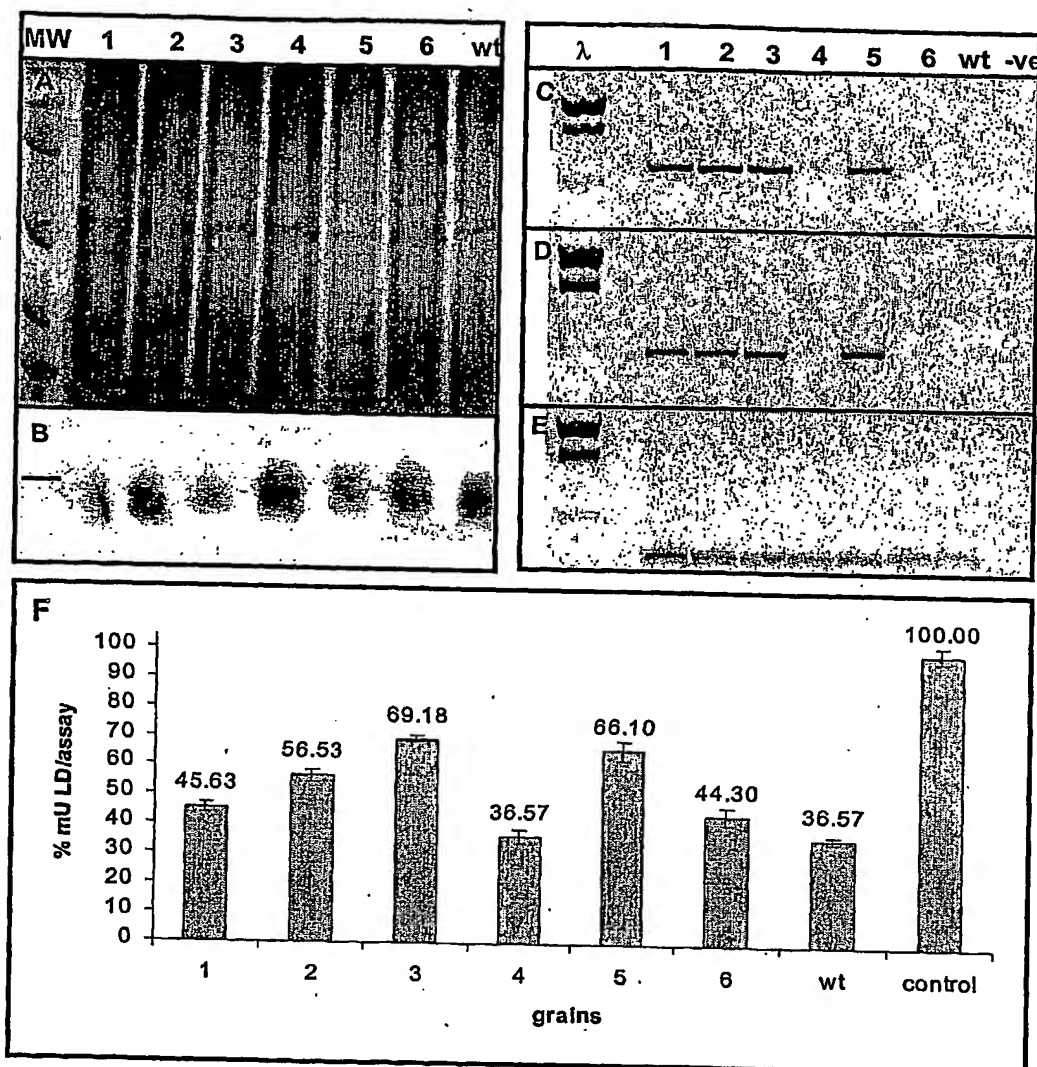


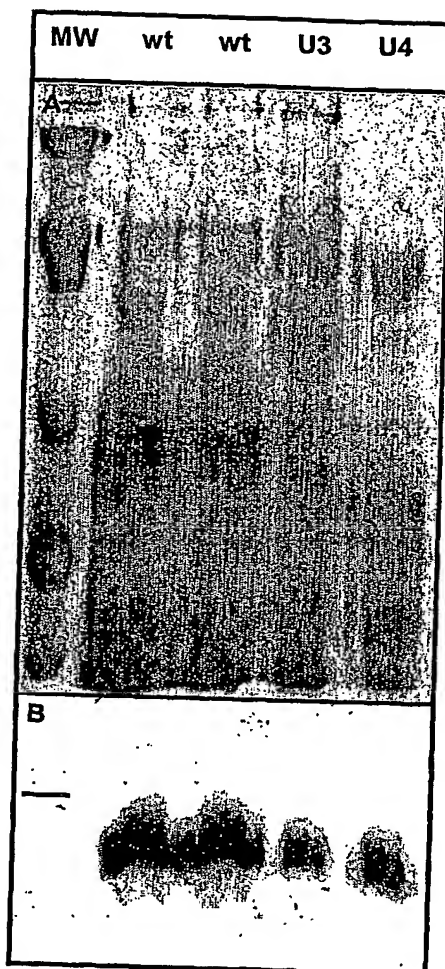
Figure 4



(A) 10% SDS-PAGE of LDI extracts corresponding to 5 μ g protein.
 (B) Immunoblot developed with antiserum against LDI; bar represents 21.5 kD.
 (C) PCR of genomic DNA with primers Inhib-6 + OCS-II for the *LDI* gene in antisense direction (817 bp).
 (D) PCR of genomic DNA primers BAR-I + BAR-II for the *bar* gene (534 bp).
 (E) PCR of genomic DNA primers TUB-F + TUB-R for the *tubulin* gene (217 bp).
 (F) LDI activity assay. The control represents the amount of LD used for each assay. LDI extracts corresponding to 10 μ g protein were mixed with LD and assayed for remaining LD activity. Each value represents the mean \pm SE of three replicate experiments.

1-6: six individual grains; wt: wildtype; MW: molecular weight marker sizes in kD are 97.4, 66.2, 45, 31, 21.5 and 14.4; λ : λ /Hind III molecular weight marker; -ve: negative control of PCR.

Figure 5



(A) 12% SDS-PAGE of LDI extracts corresponding to 5 μ g protein.

(B) Immunoblot developed with antiserum against LDI; bar represents 21.5 kD.

U3: homozygous T₂ generation transgenic line
U3; U4: homozygous T₂ generation transgenic line
U4; wt: wildtype; MW: molecular weight marker
sizes in kD are 97.4, 66.2, 45, 31, 21.5 and 14.4.

Figure 6

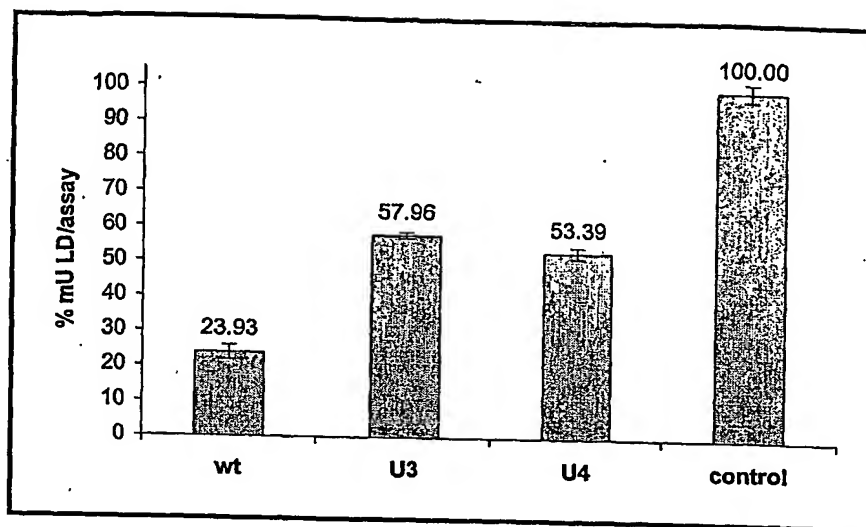
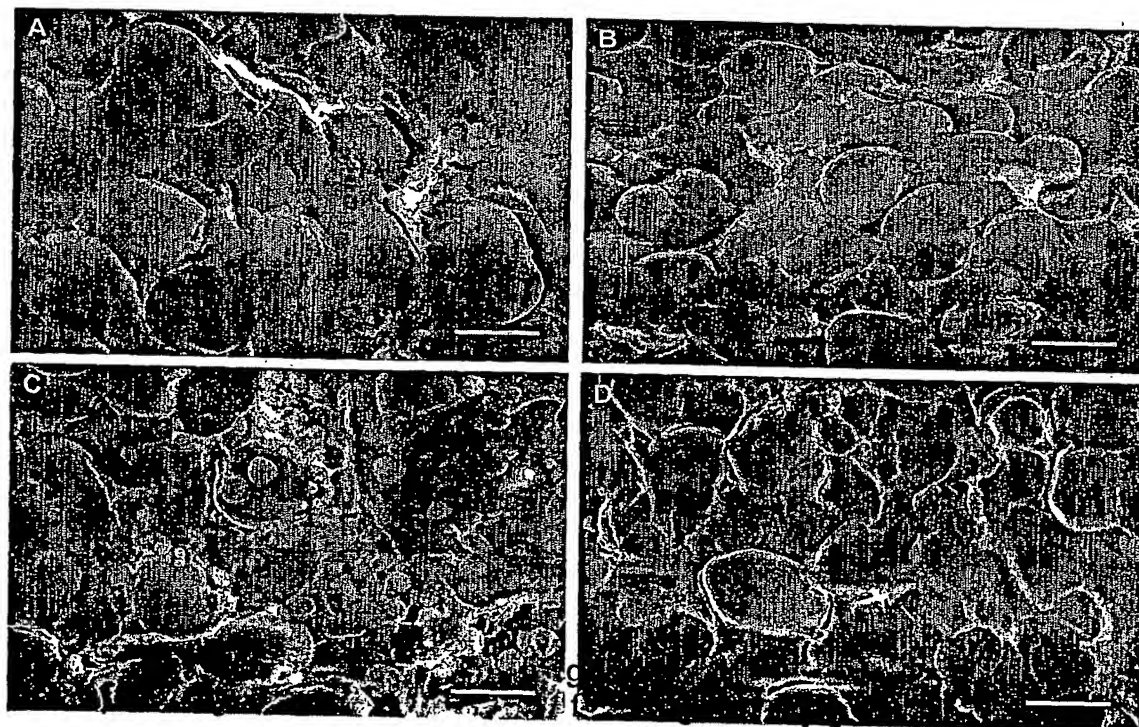


Figure 7



(A) wildtype barley grain.
 (B) transgenic T₂ generation grain of homozygous line U4.
 (C) wildtype T₁ grain of heterozygous transgenic line U3.
 (D) transgenic T₁ grain of heterozygous line U3.
 Bar is 10 μ m long.

Figure 8

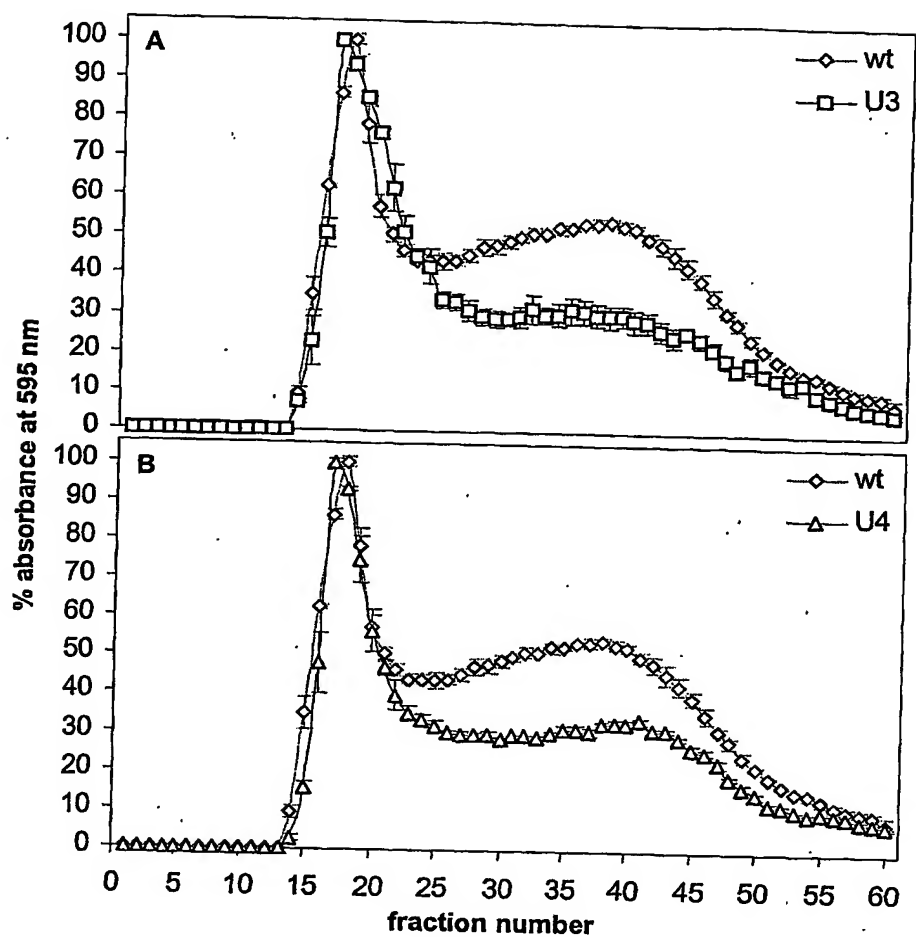


Figure 9

DNA % Identity	<i>Hordeum vulgare</i> SEQ. ID. No. 3	<i>Hordeum vulgare</i> SEQ. ID. No. 1	<i>Triticum aestivum</i>	<i>Zea mays</i>	<i>Oryza sativa</i>	<i>Hordeum spontaneum</i>
<i>Hordeum vulgare</i> SEQ. ID. No. 3	100	76.34				
<i>Hordeum vulgare</i> SEQ. ID. No. 1	98.84	100	77.27	63.45	42.35	43.09
<i>Triticum aestivum</i>			100	65.38	45.57	46.05
<i>Zea mays</i>				100	41.37	46.81
<i>Oryza sativa</i>					100	49.28
<i>Hordeum spontaneum</i>						100

% amino acid identities	<i>Hordeum vulgare</i> SEQ. ID. No. 4	<i>Hordeum vulgare</i> SEQ. ID. No. 2	<i>Hordeum spontaneum</i>	<i>Oryza sativa</i>	<i>Triticum aestivum</i>	<i>Zea mays</i>	<i>Secale cereale</i>	<i>Eleusine coracana</i>
<i>Hordeum vulgare</i> SEQ. ID. No. 4	100							
<i>Hordeum vulgare</i> SEQ. ID. No. 2	98.64	100	42.86	34.01	80.95	53.06	40.14	42.18
<i>Hordeum spontaneum</i>		43.54	100	29.92	43.54	48.98	66.67	46.26
<i>Oryza sativa</i>		33.75	25.63	100	31.88	33.13	23.75	25
<i>Triticum aestivum</i>		78.43	41.83	33.33	100	54.25	39.22	39.22
<i>Zea mays</i>		50.97	46.45	35.48	53.55	100	43.87	52.9
<i>Secale cereale</i>		48.36	79.51	29.51	50.82	55.74	100	62.3
<i>Eleusine coracana</i>		51.22	54.47	29.27	50.41	66.67	61.79	100